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Screening of Yeast from Honey for Mead Production

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Mead or honey wine is an alcoholic beverage made from honey. The main objective of this work was to evaluate the capacity of *Saccharomyces cerevisiae* strains, isolated from honey to produce mead. Nine wine yeasts were screened for ethanol, osmotic and sulphur dioxide tolerance. Seven strains from Indian rock bee honey, were evaluated in terms of their fermentation performance under ethanol, osmotic and sulphur dioxide stress. YR5 (*Saccharomyces cerevisiae*) strain from rock bee honey showed measurable growth in medium containing 10% (v/v) ethanol. They were equally sugar and SO_2 tolerant having good growth in medium containing 20% (w/v) glucose plus 20% (w/v) fructose and 250 mg/l SO_2. Based on the above results it can be concluded that a high sugar, SO_2 and ethanol tolerant *Saccharomyces cerevisiae* YR5 could be used as starter culture for commercial production of mead.

Key words: mead, yeast, ethanol, sugar, SO_2

Mead is a traditional drink, containing 8–18% (v/v) of ethanol, which results from the alcoholic fermentation of diluted honey carried out by yeasts. Mead fermentation is a time-consuming process, often taking several months and the fermentation rate depends on several factors, especially on honey variety, yeast strain and yeast nutrition (Navratil *et al*., 2001).

Honey is a natural product, mainly composed of a complex mixture of carbohydrates, minor substances such as organic acids, amino acids, proteins, minerals, vitamins, and lipids. In almost all honey types, fructose and glucose pre dominate. These two sugars account for nearly 85–95% of the honey carbohydrates (Finola *et al*., 2007).

However, when it is produced in a home made way, the beekeepers and mead producers find several problems, namely, lack of uniformity in the final product, since water content of honey changes every year, (20% maximum, except for Calluna honey which is 23%), that can induce not only refermentations by yeasts, but also metabolisation of residual sugar by acetic acid bacteria and lactic acid bacteria. This increases volatile acidity and produces abnormal esters, changing the organoleptic quality of the final product (O'Connor-Cox and Ingledew, 1991). Delayed and arrested fermentations are other problems found in mead production, causing significant delays in the marketing of mead, being sold one year after its production.

In wine production, delayed and arrested fermentations,and production of off-flavours by the yeasts, are usually associated with the inability of yeast strains to respond and adapt to unfavourable stressfull growth conditions (Attfeld, 1997; Bisson, 1999). Some possible stress factors are temperature (heat or cold) shock stresses, limitations in essential nutrients, osmotic stress, ethanol toxicity (Bauer and Pretorius, 2000). Analysis of stress resistance has been proposed as a suitable criterion for wine yeast selection (Zuzuarregui and Olmo, 2004). Yeasts used in mead production are starter yeasts, such as strains of *Saccharomyces cerevisiae* used in wine, beer and champagne production. However, regarding the composition of honey and wine must, namely the higher sugar levels (>60% versus 20– 25%) and lower nitrogen concentrations (0.04% average versus 4–5% optimum) present in honey, it was thought that these strains might not be the most suitable for mead production.

The aim of this work was to select the most appropriate yeasts isolated from honey for mead production. *S. cerevisiae* strains isolated were evaluated in terms of their fermentation performance under ethanol, sulphur dioxide, and osmotic stress. In order to characterize the yeast strains land for their suitability for mead production.

Materials and Methods

Yeast isolates from honey

In the present study, seven rock bee honey samples (fve from Andhra Pradesh, one each from Tamil Nadu and Kerala) were used for isolation of wine yeast and the isolates were designated as YR1, YR2, YR3, YR4, YR5, YR6 and YR7. One gram

of the samples from each source was inoculated into fve ml sterile yeast peptone dextrose (YPD) broth (containing per litre: glucose 20 g, peptone 10 gram, yeast extract 5 g) in test tubes. In order to prevent the fungal contaminants and to establish the fermentative abilities, surface of the broth were sealed with one cm layer of sterile paraffn and incubated at 28±1ºC for 48 h (Gupta *et al*., 1994).

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After incubation, yeast isolates were purifed from the YPD broth by dilution plate technique (Pelczar and Reid, 1958). The isolated yeast cultures were compared with standard (MTCC180) and commercial yeast strains (Baker's yeast).

Osmotic, ethanol and SO_2 stress tolerance

For the analysis of ethanol, sulphur dioxide and osmotic stress tolerance, an initial concentration of yeast of 1 X 10_5 cells/mL was used. In the all cases, fermentations were carried out with orbital agitation at 25ºC for 168 h. Yeast cell growth was followed through by measuring the optical density at 640 nm in a UV–visible spectrometer (Varian) and by counting the colony-forming units (CFU) in YPD agar. For ethanol stress tolerance, all the nine yeast strains were cultured in YPD medium containing different ethanol (Sigma–Aldrich) concentrations, *viz.,* 10, 15 and 20% (v/v). For the sulphur dioxide resistance, fermentations were carried out in YPD medium supplemented with sulphur dioxide to concentrations of 100, 250, and 500 mg/L. To induce osmotic shock, cells were transferred to YPD liquid medium containing 40% of sugars (20% (w/v) each of glucose and fructose).

In all cases, a control was maintained with yeast cells grown in YPD medium at 25ºC.

Results and Discussion

Yeast from honey

Seven yeast strains were isolated from honey samples collected from different regions in India (YR1, YR2, YR3, YR4, YR5, YR6 and YR7). All the above seven yeast isolates were screened for their stress tolerance to select suitable yeast cultures with desirable characteristics for wine fermentation. Nine yeast isolates were identifed based on their colony morphology, vegetative cell structure, budding position and pseudomycelium formation and sporulation patterns (Kreger, 1984) (Table 1). Most of the isolated yeast colonies were butyrous, raised, smooth and glossy. Few isolates (YR1, YR3, YR5 and YR6) were creamy white. These yeast isolates are large, ovoid shaped cells cells were arranged in short chain, having monopolor budding pattern and in liquid medium there is no pellicle formation with these isolates. YR2, YR4 and YR7 were cream coloured, raised and glossy. These strains are small, spherical to oval - shaped cells and these cells are arranged in cluster and in liquid medium there is an impenetrable and white pellicle formation.

The standard yeast cultures obtained from microbial type culture collection and gene bank, MTCC 180 (*Saccharomyces ellipsoideus*) and commercial yeast strains are medium sized, globose shaped cells and these cells were arranged in single and cluster, in liquid medium there is dense sediment formation and there is no pellicle formation with this isolate. Most of the isolated yeast cells are exihibit monopolor budding pattern.

The association of yeasts with many species of bees is to be expected, since bees collect nectar, an ideal fluid for yeast growth and metabolism. Bees carry yeast from the flowers to the honey reserve in the nests. Carvalho *et al*. (2010) studied the yeast species associated with honey and they observed that *Saccharomyces cerevisiae*, *Candida magnolia*, *Zygosaccharomyces mellis* and *Rhodotorula mucilaginosa* are the predominant yeast species found in honey. Twenty strains of yeast were isolated from honey and all were identifed as *Zygosaccharomyces rouxii* . Morphological and physiological properties of the strains were similar, as were some of their fermentation properties (Schneider, 1995).

Stress resistance of the selected yeasts strains

Selection of yeast type for fermentation process is an important aspect as it affects flavor and other quality parameters of the mead. Osmotic stress is an adverse condition for yeast cells that occurs at the beginning of the fermentation and more precisely in mead production, as honey has a high content on sugars (>60%). Analysis of yeast strain survival under this stress condition could provide useful information about the ability of the yeast to start growth and carry out fermentation. In order to evaluate the behaviour of the nine *S. cerevisiae* strains to osmotic stress, 20% (w/v) glucose plus 20% (w/v) fructose were added to YPD liquid medium in order to simulate as closely as possible the concentration of the sugars present in must honey. The results obtained and described in

Table 1. Characterization of yeast isolates based on cell morphology

Yeast strain	Morphological characters						
	Shape	Size	Arrangement	Budding pattern	Presence of {pseudohyphae}	Growth in liquid medium	
						Sediment formation	Pellicle formation
YR1	Ovoid	Large	Short chain	Monopolor	Absent	Signifcant	Absent
YR2	Spherical to oval	Small	Cluster	Monopolor	Absent	Signifcant	Dense & white
YR3	Ovoid	Large	Short chain	Monopolor	Absent	Signifcant	Absent
YR4	Spherical to oval	Small	Cluster	Monopolor	Absent	Signifcant	Dense & white
YR5	Ovoid	Large	Short chain	Monopolor	Absent	Signifcant	Absent
YR6	Ovoid	Large	Short chain	Monopolor	Absent	Signifcant	Absent
YR7	Spherical to oval	Small	Cluster	Monopolor	Absent	Signifcant	Dense & white
MTCC180	Globose	Medium	Short chain	Monopolor	Absent	Signifcant	Absent
Commercial yeast	Globose	Medium	Single and cluster	Monopolor	Absent	Signifcant	Absent

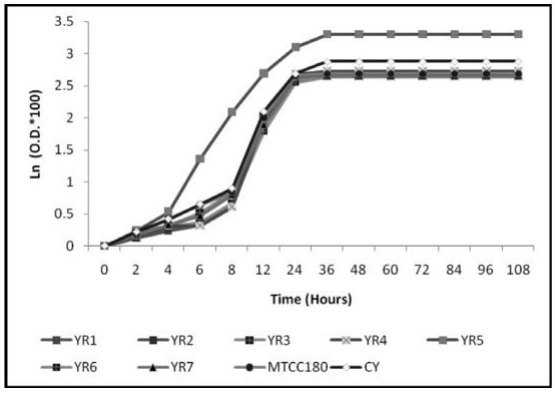

Fig. 1a. Growth as optical density (at 640nm) of the nine yeast strains subjected to osmotic stress (20% (w/v) glucose + 20% (w/v) fructose)

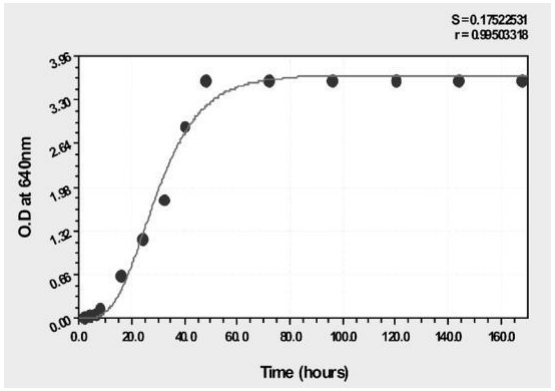

Fig 1b. Growth as optical density (at 640nm) of the YR5 yeast strain subjected to osmotic stress (20% (w/v) glucose + 20% (w/v) fructose)

Fig. 1a & b show that YR5 strain had high tolerance compare to other strain. All other strains had a similar behaviour when sugars were added. Increasing sugar concentrations for these strains prolonged their lag phases for 8 h in the media containing 20% (w/v) glucose plus 20% (w/v) fructose respectively. Also reduced growth rates were equally observed in these media. When compared YR5 with the control (Fig. 4), neither was a decrease in growth rates observed. The result was in accordance with the fndings of Pereira *et al*. (2009).

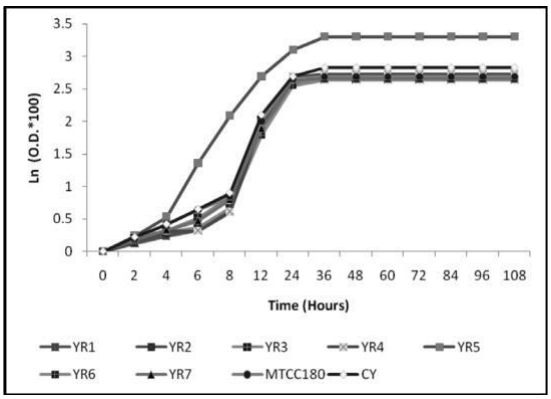

Fig. 2a. Growth as optical density (at 640nm) of the nine yeast strains subjected to ethanol stress (10%(v/v))

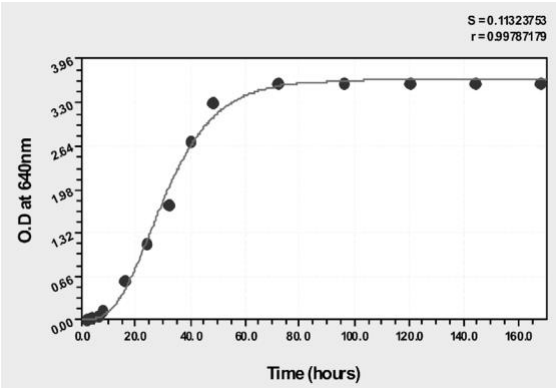

Fig. 2b. Growth as optical density (at 640nm) of the YR5 yeast strain subjected to ethanol stress (10%(v/v))

Ethanol stress is was one of the most interesting conditions to analyze, since one of the traditional criteria used to select yeast strains for production of alcoholic drinks was their tolerance to ethanol, owing to the high concentrations of this alcohol reached during fermentation. To better observe the effect of this kind of stress on cell viability, ethanol was exogenously added to the cells in a single pulse. YR5 strains showed the tolerance at 10% (v/v) ethanol (Fig. 2a & b), but there was decrease on cell viability. It was also observed that none of the stains was able to grow at concentrations of 15% (v/v) and 20% (v/v) ethanol (data not shown). Similar results were obtained by Carrasco *et al*. (2001), who observed that all commercial wine yeast strains studied were tolerant to 10% (v/v) ethanol, but most of them were significantly affected by concentrations of 12% (v/v).

Another desirable trait for fermentation of yeast strains was a high tolerance to SO_2. The growth of YR5 strains was not affected by concentrations until 250 mg/L. The presence in culture medium of SO_2 concentrations of 500 mg/L inhibited the growth of all the strains. Although the growth rate was not affected by SO_2 concentrations of 250 mg/L, there was an increase on the lag phase duration of about 8h (data not shown) (Fig 3). These results are in accordance with the ones reported by Nikolaou *et al*. (2006), who tested the resistance of six *S. cerevisiae* strains, isolated from wine must, and subjected to

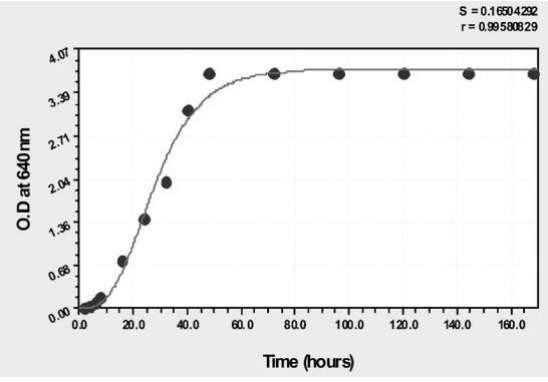

Fig. 3. Growth as optical density (at 640nm) of the YR5 yeast strain subjected to SO_2 (250ppm)

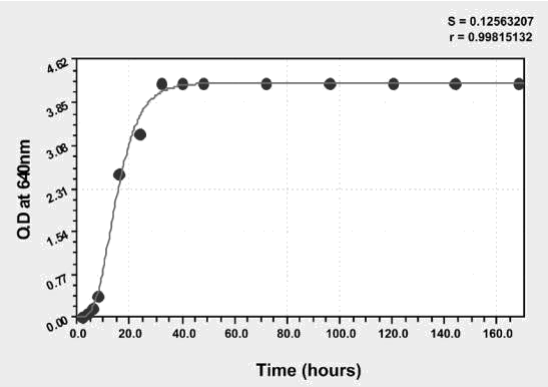

Fig. 4. Growth as optical density (at 640nm) of the YR5 yeast strain not subjected to osmotic, ethanol and SO_2 stress condition

various concentrations of sulphur dioxide (50–300 mg/L), observing that only the growth of one strain was affected by SO_2 concentrations of 300 mg/L. In all studies of our work the population growth was also confirmed by counting the colony forming unit (data not shown). Based on the results YR5 strain was selected from the seven isolated from honey. The commercial and standard yeast strain also exhibited slight stress tolerance. So these two strains also selected in order to perform posterior comparisons between the strains isolated from honey with the one used in oenology in terms of their suitability for mead production.

Thus, the yeast strain YR5 was suitable for production of wines where the desired alcohol levels were between 5 and 10%; while standard and commercial yeast strain could be used where the desired level is between 4 and 7%. The isolation of high ethanol-tolerant and sugar-tolerant yeast strains from rock bee honey revealed the need to look further into other honey types which might yield new strains of wine yeasts.

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